Interference Between Inactivated Bacterial Virus and Active Virus of the Same Strain and of a Different Strain*

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Received June 6, 1942

Introduction

In the preceding paper (1) a case of interference between two bacterial viruses (Bacteriophages), α and γ, which are active on the same bacterial strain, has been described. Each bacterium infected with both viruses liberates only virus γ; virus α is suppressed. One particle of virus γ is sufficient to suppress the growth of virus α in a bacterium. With mixed infection, the growth of virus γ on the bacteria is normal, unless virus α is present in very great excess. Only when virus γ reaches the bacteria several minutes later than virus α does the latter succeed in growing in some of the bacteria to the exclusion of the former.

The ability of virus γ to interfere with the growth of virus α represents a novel property of bacterial viruses. The present paper is concerned with attempts to dissociate the interfering from the reproducing capacity of the virus.

Material and Methods

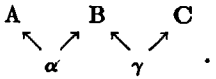
Most of the material and methods used in these experiments have been discussed in detail in the preceding paper (1).

The *material* consisted of two bacterial viruses, α and γ, both active on the same host, B (*Escherichia coli*). Each of them is active also on one of two bacterial

* This work was supported by grants-in-aid from the Dazian Foundation for Medical Research and from the Research Fund of Vanderbilt University.

‡ Fellow of the John Simon Guggenheim Memorial Foundation.

indicator strains, A and C, as shown in the following scheme, where arrows indicate susceptibility:



When either virus α or virus γ is grown separately, in the presence of bacteria of the strain B, the average yield of virus is 135–140 particles per infected bacterium, liberated at the moment of lysis. The minimum latent period is 13 minutes for virus α and 21 minutes for virus γ.

Methods. For the thermal inactivation, virus samples were put in a water bath of the desired temperature, withdrawn at intervals, and tested for the remaining virus activity.

For the inactivation with ultraviolet rays, the total radiation of a mercury lamp was used. The virus was exposed under standard conditions, and samples were removed from time to time. Absolute absorption doses (not given in this paper) would be of no significance, since, the virus being exposed in crude suspension, foreign material is largely responsible for the absorption.

For the growth experiments, the previously described "one-step growth" was used. Experiments for testing the interfering activity of variously treated samples of virus γ were generally made under standard conditions. Constant amounts of bacteria and virus α were mixed with the treated virus γ, and the growth of virus α was followed. Most experiments were run in parallel with a similar control: the sample of treated virus γ to be tested was replaced by the same amount either of broth, or of normal virus γ taken from the stock from which the treated sample came. Under standard experimental conditions, and in the absence of virus γ, the titer of virus α increases by a factor of 35–45 during the step of growth. The step begins 13 minutes and is completed 20–25 minutes after adding virus α to the bacterial culture. The reduction of this increase can be used as a measure of the amount of suppressing activity. Special experiments will be described in the next section.

Experimental Results

A. Heat Inactivated Virus

Samples of virus γ, inactivated at a temperature of 55°C. until their titer (initially about 10^{10} particles/cc.) was reduced to 10^3 or less, were tested for interfering activity. The growth of virus α in the presence of heat inactivated virus γ, was always found to be normal. This shows that heat inactivated virus γ loses its interfering as well as its reproducing capacity.

B. Ultraviolet Inactivated Virus

I. Suppression of the Growth of Virus α by Ultraviolet Inactivated Virus γ

It was thought that inactivation by ultraviolet radiation would offer a favourable chance of conserving the interfering activity of the virus. It is known that ultraviolet inactivated viruses are not so deeply altered that they lose their antigenic power, or their ability to act as vaccines (2, 3, 4).

The first experiments, with samples of virus γ completely inactivated by irradiation, showed immediately that this virus had maintained at least part of its ability to interfere with the growth of virus α. In a typical experiment, virus α increased six times, as compared with 35 times in the control experiment without virus γ. Controls with irradiated broth and bacterial filtrates never inhibited the growth of virus α. Following these preliminary results, different experiments were devised to study the dependence of the interfering activity on various factors.

TABLE I

The Growth of Virus α in the Presence of Virus γ Which Has Been Irradiated for Various Times

Experiment	Time of irradiation of virus γ	Residual titer of virus γ	Dilution of virus γ in the adsorption mixture	Increase of the titer of virus α
No.	*minutes*	*virus/cc.*		
53b	0	1.2×10^{10}	1.5/10	1.2 times
53a	5	3.5×10^{4}	1.5/10	2 "
64b	10	0	0.5/10	10 "
49	15	0	1.0/10	11 "
59	60	0	1.5/10	45 "
64c	Control (no virus)			40 "

1. *Different Irradiation Times.* Results are shown in Table I. The small amount of virus γ remaining in the less irradiated sample (5 minutes) is not sufficient to interfere with the growth experiments, as it can infect less than 1/10,000 of the bacteria.

It is seen that the suppression of the growth of virus α, which is practically complete with normal virus γ, is almost complete with virus γ irradiated five minutes; it diminishes progressively with irradiation times of 10 and 15 minutes, and is totally absent with virus irradiated for 60 minutes.

The virus α-suppressing activity of suspensions of virus γ is, therefore, progressively destroyed by increasing doses of ultraviolet rays. This activity is, however, much more resistant than the reproducing activity of the virus, since to destroy it requires much larger doses.

2. *Proof That the Inhibiting Action of Irradiated Suspensions of Virus γ Is Due to the Inactive Virus.* The interference between virus γ and virus α is strictly quantitative: every cell infected by one particle of virus γ becomes unable to reproduce virus α. Therefore, by increasing amounts of virus γ, more bacteria are infected, and virus α is more completely suppressed. It was desired to prove that the virus α-suppressing activity of irradiated virus γ suspensions followed the same quantitative relationship.

An experiment was performed, in which various amounts of irradiated virus γ were added to different portions of a mixture of bacteria and virus α, and the growth of virus α was followed.

Experiment No. 75. 1.0 cc. of a bacterial culture was introduced in each of three tubes. Tube 1 was inoculated with 0.01 cc. of inactivated virus γ (irradiated five minutes) + 0.005 cc. of virus α. Tube 2 was inoculated with 0.04 cc. of inactivated virus γ + 0.005 cc. of virus α. Tube 3 was inoculated with 0.1 cc. of inactivated virus γ + 0.005 cc. of virus α. After 4.5 minutes, the three mixtures were diluted and the growth of virus α followed.

Tube	Amount of irradiated virus γ	Increase of the titer of virus α
1	0.01 cc.	61 times
2	0.04 cc.	26 times
3	0.10 cc.	5 times

It is evident that the inhibition of the growth of virus α diminishes with the amount of the irradiated virus γ present. Although the experimental data do not permit a complete calculation, the amount of virus α growth in the three different tubes is that expected, according to the hypothesis that virus α does not grow in those bacteria which have adsorbed at least one particle of virus γ.

We conclude that the virus α-suppressing activity of irradiated suspensions is due to infection of the bacteria with the particles of inactivated virus γ ("partially inactivated particles").

3. *Proof That the Inactivated Virus Particles Are Adsorbed by the Bacteria.* The inhibition of virus α growth by inactivated virus γ offered a possibility of testing and measuring the adsorption of the partially inactive particles by the bacteria. If they are adsorbed by the bacterial cells, the interfering activity of a suspension should be reduced by "extracting" it with bacteria. The following experiment proves that the partially inactivated virus γ particles can be extracted with sensitive bacteria.

Experiment No. 64. 0.8 cc. of a bacterial culture was inoculated with 0.2 cc. of inactivated virus γ (irradiated ten minutes). The mixture was kept twenty minutes at 37°C., then centrifuged ten minutes. The supernatant was removed and tested for inhibiting activity upon virus α growth. Two controls were run: in one, the supernatant was replaced by broth, in the other by a corresponding amount of irradiated virus γ not previously extracted with bacteria.

Tube	Content	Increase of the titer of virus α
1	Bacteria + supernatant + virus α	32 times
2	Bacteria + irradiated virus γ + virus α	10 times
3	Bacteria + broth + virus α	38 times

The suspension extracted with bacteria shows very little interfering activity, while the control with irradiated virus γ not previously extracted with bacteria shows a definite inhibition of virus α growth. A rough calculation shows that the extraction has reduced the interfering activity to about one-tenth. This is in quantitative agreement with the known adsorption rate of virus γ by bacteria.

II. The Inhibition of Bacterial Growth by Ultraviolet Inactivated Virus γ

Although unable to reproduce itself on the sensitive bacteria, the partially inactivated virus γ might lyse the bacteria. This possibility was excluded by microscopic observation on agar of bacteria after treatment with inactive virus, which showed that these bacteria do not undergo lysis. However, the observation also showed that they do not divide, and remain unchanged for hours. Whether or not bacteria dividing at the moment of infection can carry this division step to completion, could not be decided, because microscopic observation always starts several minutes after bacteria and virus are mixed.

Suspensions of virus γ irradiated with very large doses of ultraviolet rays, enough to completely destroy their interfering activity, do not appear to inhibit the bacterial growth. This point, however, has been studied only qualitatively.

In order to study more closely the inhibition of bacterial growth, experiments were done, in which bacteria and partially inactivated virus γ were mixed, and the survival of bacteria studied by colony count. The number of colonies decreased progressively with increasing amounts of irradiated virus.

Table II shows an experiment in which various amounts of irradiated virus γ were mixed with equal amounts of bacteria. The mixtures were

diluted after five minutes, and the surviving bacteria counted by plating for colony count. The number of surviving bacteria diminishes as the amount of irradiated virus increases. If the growth of a bacterium is prevented by adsorption of one particle of virus, one should expect the fraction of surviving bacteria in the different mixtures to follow Poisson's formula:

$$\text{surviving bacteria} = e^{-n},$$

where n is the unknown number of virus particles adsorbed per bacterium (multiplicity of infection). The values of e^{-n} in the different mixtures are experimentally determined, and the values of n thus obtained should be proportional to the concentration of irradiated virus. In other words, the ratio of the values in Column 4 to those in Column 2 should be constant. This ratio is listed in Column 5. The result agrees with the theoretical expectation. We conclude, that a bacterium is inhibited from growing whenever it has adsorbed at least one partially inactive particle of virus γ. This experiment permitted us to calculate that our virus sample, after five minutes irradiation, contained 4×10^9 partially inactive particles of virus γ/cc. Since the original stock before irradiation contained 1.2×10^{10} particles of active virus/cc., we conclude that with such a dose of radiation about two-thirds of the virus have been completely destroyed.

TABLE II

Survival of Bacteria after Treatment with Different Amounts of Irradiated Virus γ

Experiment No. 79. Different amounts of virus γ irradiated for five minutes were added to different portions of a bacterial suspension. After five minutes the mixtures were diluted, and dilutions plated for bacterial colony count.

Tube	Concentration of irradiated virus relative to Tube 1	Surviving bacteria	Multiplicity of infection (calculated by Poisson's formula)	Multiplicity Virus concentration
		per cent		
1	1	0.26	5.95	5.95
2	0.5	5	2.99	5.98
3	0.3	15	1.90	6.33

III. The Interference of Partially Inactive Virus γ with Active Virus γ

In the preceding paper (1), it was shown that single or multiple infection of a bacterium with the same virus produces no difference in the final yield of virus particles per bacterium. To explain this fact, the existence of "self-interference" was proposed. Accordingly, only one virus particle is able to reproduce itself in each bacterium. Both "self-interference" and "γ versus α interference" were tentatively explained as competitive blockade of a key-enzyme present in the cell in limited amount. If this hypothesis is correct, ultraviolet inactivated virus γ, which can still interfere with the growth of virus α, should also be able to interfere with the growth of normal virus γ.

To test this possibility, irradiated virus γ was put into the presence of bacteria at various intervals of time before or after the active virus γ. Table III shows the results.

The growth of active virus γ is strongly reduced in the presence of inactivated virus γ. This reduction is more evident when the active virus reaches the bacteria several minutes after the inactive, and conversely, it almost disappears when the active virus is given a few minutes precedence. These results prove that there is interference between inactive and active virus γ. The fact that the amount of interference depends on the order in which the two reach the bacteria strongly supports the idea of a mechanism of interference by blockade of a bacterial constituent.

TABLE III

Suppression of the Growth of Virus γ by Irradiated Virus γ

Experiment	Amount of irradiated virus γ	Interval between the introduction of irradiated virus γ and of normal virus γ	Amount of growth of virus γ in per cent of the growth occurring in the absence of irradiated virus γ
No.		*minutes*	*per cent*
73a	1.5/10	+4	20
72b	1.5/10	+1.5	35
73b	1.5/10	−2	>90
18	0		100

IV. The Relation between the Inhibition, by Partially Inactivated Virus γ, of Bacterial Growth, α-Growth and γ-Growth

Ultraviolet inactivated virus γ has been shown above to interfere with the growth of the bacteria, and with the growth of both virus α and virus γ on the bacteria. To investigate the quantitative relationship

between these three actions, experiments were done in which the suppression of bacterial growth by inactive virus γ was compared under identical conditions with the inhibition of the growth of viruses α and γ.

Experiment No. 72. A bacterial culture, after being assayed for bacterial titer, was divided in three portions (Tubes 1, 2, 3). At time zero, to each tube 15 volume per cent of inactivated virus γ (irradiated five minutes) was added. After 1.5 minutes, 0.5 volume per cent of virus α was added to Tube 2, and 0.5 volume per cent of active virus γ was added to Tube 3. After 6.5 minutes, the three mixtures were diluted. Dilutions from Tube 1 were plated for bacterial count. The growth of virus α and of virus γ was followed in the dilutions from Tubes 2 and 3. The amount of growth of the viruses was compared with the growth expected in the absence of irradiated virus γ.

Tube	Content	Result
1	Bacteria + inactive virus γ	Bacterial count reduced to 0.85 per cent
2	Bacteria + inactive virus γ + virus α	Growth of virus α reduced to 0.75 per cent
3	Bacteria + inactive virus γ + virus γ	Growth of virus γ reduced to 35 per cent

The results of this experiment show that the inhibition of bacterial growth and of virus α growth are approximately the same, whereas the inhibition of γ growth is much less complete.

This difference cannot be due alone to the fact that some bacteria become infected with active virus γ earlier than with inactive virus γ. In the experiment number 73a (see Table III) the inactive virus could infect practically all cells before the introduction of the active virus; yet the latter grew in a relatively high percentage of the bacteria. It appears, then, that active virus γ can grow in some of the bacteria infected with ultraviolet inactivated virus γ, although these bacteria have lost the capacity, both of reproducing themselves and of allowing the growth of virus α.

V. Partially Inactive Virus Does not Reproduce in the Bacteria

It was possible that bacteria infected with ultraviolet inactivated virus γ might liberate virus γ in the partially inactive form, although they do not undergo lysis. In this case, the newly formed virus, being deprived of the capacity of lysing bacteria, would not be detected by the usual plaque counts. On the other hand, the interfering activity of the irradiated virus γ should be found to increase in the presence of bacteria.

Filtrates from suitable mixtures of bacteria and irradiated virus γ, and from their sub-cultures, were tested for interfering activity and were always found to lack it. Therefore, we conclude that irradiated virus cannot reproduce itself on the bacteria in the partially inactive form.

VI. Ultraviolet Inactivated Virus α

Suspensions of virus α, inactivated by ultraviolet rays, were found to exert no inhibiting activity, either on bacterial growth, or on the growth of either virus α or γ. It is, therefore, impossible even to decide whether this inactive virus is still adsorbed by the bacterial cells.

Discussion

Suspensions of a bacterial virus γ, after inactivation by ultraviolet rays, may conserve the ability to interfere with the growth of another virus α acting on the same host. The interfering activity is present only in irradiated suspensions of virus γ, not in bacterial filtrates, or in irradiated suspensions of virus α. It is destroyed by heat together with the virus activity, and it is adsorbed by bacteria at the same rate as the active virus γ. Finally, the amount of virus α-suppressing activity is in such quantitative relation with the amount of inactive virus present as to prove that the adsorption of one inactive particle of virus γ on the bacterial cell is sufficient to inhibit the growth of virus α. We conclude that irradiated particles of virus γ, although they have lost their reproducing activity, may keep their ability to interfere with the growth of virus α (partially inactive particles).

The ultraviolet inactivated virus γ also shows the capacity of inhibiting the growth of the sensitive bacteria. These are not lysed, but deprived of the ability to divide. The number of bacterial cells thus affected corresponds to that of the cells in which the growth of the virus α is inhibited; the two actions evidently are manifestations of the same phenomenon. The suppression of bacterial growth must be due to inhibition by virus γ of some fundamental step in the synthetic processes of the bacterial life cycle. With normal virus γ, this inhibition is not observable because of the lysis of the cell; with the inactivated virus, which has lost the lysing capacity, the inhibition becomes apparent.

The growth of normal virus γ, also, can be inhibited by the previous action of ultraviolet inactivated γ on the cells. However, this suppression is not as extensive as the inhibition of the growth of virus α.

Altogether, the experiments described above lend support to the hypothesis (1) that the interference between viruses γ and α is due to competition for some material, probably of enzymatic nature, necessary for virus reproduction and present in limited amount in the cell. Virus γ, either normal or partially inactivated by ultraviolet radiation, is capable of combining with this material to the exclusion of virus α. Inactive virus γ can also, to a certain extent, exclude active virus γ from this material. The suppression of bacterial growth by partially inactive virus γ further suggests that the blocked enzyme is intimately connected with the mechanism of bacterial division.

The interesting experiments of Andrewes and Elford (5) on the "killing" of bacteria by virus in the presence of sodium citrate may be quoted in support of our interpretation of the bacteriostatic action of irradiated virus. These authors found that in the presence of citrate the growth of the bacteria is instantly inhibited by the addition of virus, although the virus does not grow and the cell is not lysed. The citrate, by precipitating the calcium ions, prevents virus growth but not virus adsorption. The analogy between this and our present case lies in this: in both cases virus is adsorbed but unable to grow and in both cases bacterial growth stops almost instantly.

The interfering ability of virus γ is more resistant to irradiation than is the reproducing property, since it is present when the latter is destroyed. Nevertheless, the interfering property, too, is progressively destroyed by increasing doses of irradiation.

The inactivation of bacterial viruses and of other viruses by ultraviolet and x-radiation has been proved to be of a direct type (6, 7, 8, 9, 10). It has been shown to result from the absorption of radiation within the virus particle. Therefore, the smaller the volume to be hit by radiation, the more resistant is the virus particle. The fact that the interfering activity of virus γ can stand larger doses of radiation than the reproducing capacity suggests that its suppression requires, either an extensive damage to the virus particle by multiple acts of absorption, or the destruction of a different part (enzymatic property?) of the particle.

Our results show the possibility of rendering bacterial cells insensitive to viruses by treatment with ultraviolet inactivated virus. An extension of this possibility to the field of plant and animal viruses brings us to consider the production of ultraviolet vaccines. Ultraviolet irradiated rabies virus has been successfully used by Hodes, Webster and Lavin (3) and by Webster and Casals (4) as anti-rabies vaccine. Jungeblut and Sanders (11) mention the fact that a murine strain of poliomyelitis virus, after ultraviolet irradiation, may retain the ability to interfere with the normal monkey strain.

Results obtained with bacterial viruses may not be applicable in their entirety to the case of viruses acting on more complex hosts. They may bear more similarity to plant than to animal viruses, since immunity and interference, in plants as in bacteria, are known to be strictly cellular.

Our results would suggest that in order to be used as a cell protecting vaccine, a virus should receive the *minimum* dose of radiation sufficient to destroy its infectivity, because the protecting activity is itself slowly destroyed by the radiation. Since partially inactivated virus cannot reproduce itself, the vaccine should be used in amounts large enough to block most or all of the "spots" in which active virus could grow (sensitive cells or cell components).

Although the situation in the case of animal viruses is complicated by the occurrence of serological mechanisms of immunity (12), it is possibly not meaningless that the results obtained with ultraviolet irradiated anti-rabies vaccine (3, 4) seem to be in agreement with the above suggestions.

Summary

1. It is shown that a bacterial virus, γ, after inactivation by ultraviolet radiation, retains its ability to interfere with the growth of another virus, α, acting upon the same host. A *single* partially inactivated particle is sufficient to suppress the growth of virus α in one bacterium.

2. The partially inactivated virus γ is adsorbed by the sensitive bacteria, and it inhibits their growth without producing lysis.

3. The partially inactivated virus γ interferes also with the growth of active virus γ.

4. The interfering activity of virus γ, although more resistant to radiation than the reproducing activity, is progressively destroyed by larger doses of ultraviolet rays.

5. These results are interpreted as supporting the hypothesis that interference between bacterial viruses is due to competition for a "key-enzyme" present in limited amount in each bacterial cell. They suggest that this enzyme is also essential for the bacterial growth.

6. The bearing of these results on the problem of anti-virus vaccines produced by irradiation is discussed.

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